REMARKS

Claims 1-17 were pending. Applicants have amended Claims 1-3, 7, 9-14 and 17, without narrowing their scope and added new Claims 18-37. A marked-up version of Amended Claims 1-3, 7, 9-14 and 17 is attached hereto as Exhibit B. A clean copy of all pending claims is attached hereto as Exhibit C.

I. THE AMENDMENT OF THE SPECIFICATION

The specification has been amended in paragraph 194 of U.S Publication No. 2002/0058262 A1, to replace incorrect formula 6 with correct formula 6. As the amendment to the specification is fully supported by the specification as originally filed, for example at paragraphs 192, 184 and formula 4, it does not constitute new matter. Entry thereof is therefore respectfully requested.

II. THE AMENDMENT OF THE CLAIMS

The claims have been amended to correct minor formal errors. Claims 1, 7-10 have been amended to change the verbs to the active voice. For example, phrases like "is amplified" (from Claim 1) or "amplication of" (from Claims 7-10) have been changed to "amplifying." Claims 9 and 10 have also been amended to correct minor formal errors in order to clarify what Applicants regard as the invention. Claims 2 and 3 have been amended to remove redundant steps (a)-(d) and to correct minor formal errors in order to clarify what Applicants regard as the invention. Claim 12 has been amended in order to correct minor grammatical and spelling errors. Claims 13 and 17 have been amended to capitalize trademarks. No new matter has been added.

Applicants emphasize for the record that none of the amendments made herein are narrowing amendments made to overcome any "prior art" under 35 U.S.C. §§ 102 or 103. Applicants expressly reserve the right to equivalents of all claim limitations to the full extent available.

New Claims 18-37 are fully supported in the specification and in the Claims as originally filed. Support for new Claims 18-26 can be found in the specification at, for example, the bottom of page 4 through the end of page 5 as well as from the beginning of page 11 through the bottom of page 12 (paragraphs 20-28 and 69-83 of U.S Publication No.

2002/0058262 A1). Additionally, new Claims 18-23 are supported by original Claims 1-6, respectively. Support for new Claims 24-26 can also be found in original Claim 6.

New Claims 27-30 are supported in the specification at, for example, the middle of page 6 through the top of page 7, the top of page 13 through the middle of page 15 and the top of page 19 through the middle of page 26 (paragraphs 35-44, 85-101 and 122-187 of U.S Publication No. 2002/0058262 A1). Additionally, new Claims 27 and 28 are supported by original Claim 7 and new Claims 29 and 30 are supported by original Claim 8.

Support for new Claims 31-33 can be found in the specification at, for example, the bottom of page 26 through the middle of page 30 (paragraphs 188-221 of U.S Publication No. 2002/0058262 A1). Support for new Claim 29 can also be found in the specification at, for example, the middle of page 7 through the top of page 8, the last paragraph on page 15, from the beginning of page 31 through the middle of page 34 (paragraphs 45-54, 102 and 225-244 of U.S Publication No. 2002/0058262 A1) and in Claim 10, as originally filed. New Claims 30-31 are also supported by original Claim 11. The terms F_t and F_r used in new Claim 29 are supported fully in the specification, at for example, paragraphs 50, 206, 216 and 225 to 229 of U.S Publication No. 2002/0058262 A1, which refer to functions for the target nucleic acid and the reference nucleic acid. In addition, the terms (Cp-Tar) and (Cp-Ref) refer to Cp values for the target and reference nucleic acids in the sample to be analysed and the terms ($Cp\text{-}Tar_{cal}$) and ($Cp\text{-}Ref_{cal}$) refer to Cp values for the target and reference nucleic acids in the calibrator sample and are fully supported fully in the specification, at for example, paragraph 228 of U.S Publication No. 2002/0058262 A1. Terms such as F_T(Cp-Tar), F_R(Cp-Ref), $F_T(Cp\text{-}Tar_{cal})$ and $F_R(Cp\text{-}Ref_{cal})$ refer to the value of the functions F_t and F_r at the cp values (Cp-Tar), (Cp-Ref), ($Cp\text{-}Tar_{cal}$) and ($Cp\text{-}Ref_{cal}$) and have been used to clearly, conveniently and succinctly claim the invention. These terms do not constitute new matter and are fully supported in the specification, at for example, paragraphs 52-53, 208-209, 218-219 and 228-229 of U.S Publication No. 2002/0058262 A1.

Support for new Claims 34-37 can be found in the specification at, for example, the bottom of page 17 through the top of page 19 (paragraphs 112-120 of U.S Publication No. 2002/0058262 A1). Additionally, new Claims 34 and 35 are supported by original Claims 12 and 13, respectively, and new Claims 36 and 37 are supported by original Claim 14.

III. THE OBJECTION TO THE SPECIFICATION

Claims 14 and 17 are objected to because the trademark SYBR® Green I allegedly should be capitalized and should be accompanied by generic terminology. Applicants submit that the objection to Claim 14 is moot because the trademark does not appear in Claim 14.

The use of a trademark is permitted in a patent application if the product to which the trademark refers is set forth in such language that its identity is clear and if it is distinguished from common descriptive nouns by capitalization. *See* MPEP § 608.01(v). Applicants submit that upon entry of the instant amendment the term SYBR® Green I in Claim 17 is properly capitalized in accordance with MPEP § 608.01(v).

Applicants further submit that Applicants cannot provide the generic terminology for SYBR® Green I because the full chemical name and/or the structure of SYBR® Green I is not publicly available. Nevertheless, Applicants submit that the identity of SYBR® Green I would be clear to one of skill in the art because is is commercially sold under the name SYBR® Green I and described in patent and scientific publications under the name SYBR® Green I. In view of the foregoing, Applicants submit that SYBR® Green I is properly capitalized and is clearly identified as required by MPEP § 608.01(v). Therefore, Applicants respectfully request that the objection to the specification be withdrawn.

IV. THE REJECTIONS UNDER 35 U.S.C. § 103

Claims 1-17 stand rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Wittwer *et al.*, 1997 (WO 97/46707, "Wittwer") in view of Brown *et al.*, 2000 (U.S. Patent No. 6,143,496, "Brown"). Applicants traverse these rejections on the grounds that the references cited by the PTO are not sufficient to establish a *prima facie* case of obviousness against any of the Claims.

A. The Legal Standard

To reject claims in an application under 35 U.S.C. § 103, the Patent Office bears the initial burden of establishing a *prima facie* case of obviousness. *In re Bell*, 26 USPQ2d 1529, 1530 (Fed. Cir. 1993); MPEP § 2142. In the absence of establishing a proper *prima facie* case of obviousness, applicants who comply with the other statutory requirements are entitled

to a patent. *In re Oetiker*, 24 USPQ2d. 1443, 1444 (Fed. Cir. 1992). In order to establish *prima facie* obviousness, three basic criteria must be met.

First, the prior art must provide one of ordinary skill in the art with a suggestion or motivation to modify or combine the teachings of the references relied upon by the PTO to arrive at the claimed invention. When an obviousness determination relies on one reference, there must be suggestion or motivation to modify the teaching of the reference in the manner suggested by the PTO. *In re Grabiak*, 226 USPQ 870 (Fed. Cir. 1985). Alternatively, when an obviousness determination relies on a combination of two or more references, there must be some suggestion or motivation to combine the references. *WMS Gaming Inc. v. International Game Technology*, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999). The suggestion or motivation to combine the references generally arises in the references themselves, but may also be inferred from the nature of the problem or occasionally from the knowledge of those of ordinary skill in the art. *See id.* The mere fact that references could be modified or combined does not render the resultant modification or combination obvious unless the prior art also suggests the desirability of the modification or combination. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Second, the prior art must provide one of ordinary skill in the art with a reasonable expectation of success. Thus, the skilled artisan, in light of the teachings of the prior art, must have a reasonable expectation that the modification or combination suggested by the PTO would succeed. *In re Dow*, 5 USPQ2d 1529, 1531-32 (Fed. Cir. 1988).

Third, the prior art, either alone or in combination, must teach or suggest each and every limitation of the rejected claims. *In re Gartside*, 53 USPQ2d 1769 (Fed. Cir. 2000). The teaching or suggestion to make the claimed invention, as well as the reasonable expectation of success, must come from the prior art, not Applicants' disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). If any one of these criteria are not met, *prima facie* obviousness is not established, and Applicants are not required to show new or unanticipated results. *In re Grabiak*, 226 USPQ 870 (Fed. Cir. 1985).

B. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of Claims 1-8 and 15

Applicants respectfully submit that the references cited by the PTO are not sufficient to establish a *prima facie* case of obviousness against Claims 1-8 and 15 because neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of Claims 1-8 and 15. As discussed in Section A above, in order to establish *prima facie* obviousness, the PTO must show that the prior art, either alone or in combination, teaches or suggests each and every limitation of the rejected claims. By failing to show such a teaching or suggestion in the art, the PTO has failed to establish *prima facie* obviousness against Claims 1-8 and 15.

Independent Claim 1 recites a method of determining amplification efficiency of a target nucleic acid as a function of the original amount of target nucleic acid where, *inter alia,* a defined signal threshold is set and a cycle number where amplification, as measured in real-time, exceeds the threshold is determined for each dilution in a series. Claims 2-8 and 15 depend from Claim 1.

Wittwer does not teach each and every element of Claims 1-8 and 15. As acknowledged by the PTO, Wittwer does not teach preparation of a dilution series of the target nucleic acid. In addition, Applicants submit that Wittwer does not teach a method of determining amplification efficiency *as a function of the amount of target nucleic acid*, nor does it teach a method of determining amplification efficiency based on the determination of the cycle number at which a defined signal threshold value is exceeded.

Contrary to what the PTO cites, Wittwer does not teach a method for determining the efficiency of the amplification of a target nucleic acid as a function of the original amount of target nucleic acid. The PTO cites the last line of the Wittwer Abstract as well as Figures 22, 45, 46 and 59 as teaching a method for determining amplification efficiency. *See* p. 7, last paragraph of the instant Office Action.

Applicants submit that the Wittwer Abstract teaches or suggests nothing about determining amplification efficiency. The last line of the Wittwer Abstract recites that "[t]he present invention provides that fluorescence *monitoring* of PCR is a powerful tool for DNA quantification." *See* Wittwer, Abstract (emphasis added). The Abstract teaches *monitoring* of PCR; not determining amplification efficiency.

The PTO also cites Figure 22 as teaching a method for determining amplification efficiency. However, Figure 22 does not teach a method for determining the efficiency of the amplification of a target nucleic acid. Figure 22 shows curves for the amplification of 10^4 and 10^5 copies of a purified template (standards) compared to the amplification of genomic DNA containing "slightly more than 10^4 copies of the target." *See* Wittwer, page 57, lines 25-30. Figure 22 teaches endpoint interpolation to compare the fluorescence of the genomic DNA with the standards at a given cycle number and threshold interpolation to determine the cycle number at which each curve exceeds a given fluorescence value. *See* Wittwer, page 57, line 32 through page 58, line 4. As mentioned explicitly therein, Wittwer teaches two methods of *interpolation* of data (*see* Wittwer, page 58, lines 5-6) to quantitate the amount of the genomic DNA sample, not a method for determining amplification efficiency as a function of the original amount of target nucleic acid. Furthermore, as discussed in more detail below regarding page 59 of Wittwer, Wittwer's endpoint interpolation methods require that amplification efficiency be constant, *i.e.* not dependent on the amount of nucleic acid. Since Wittwer teaches that amplification efficiency is constant and not dependent on the amount of a nucleic acid, Figure 22 of Witter teaches away from a method of determining amplification efficiency as a function of the amount of a target nucleic acid as recited in Claim 1.

The PTO also alleges that Figures 45 and 46 teach a method for determining amplification efficiency. Contrary to what the PTO cites, Figures 45 and 46 have nothing whatsoever to do with amplification efficiency; instead, they describe competitive quantitative PCR. Figure 45 shows the melting curve obtained when two purified PCR products differing in T_m by about 2 °C are mixed and Figure 46 is a plot showing how the relative amount of each PCR product can be quantified. *See* Wittwer, page 18, lines 1-5 and page 81, lines 23-28.

The PTO also contends that Figure 59 teaches the determination of amplification efficiency. Figure 59 does not teach the determination of amplification efficiency. Instead, Figure 59 teaches a correction method for abnormal or unexpected amplification of unintended nucleic acid (such as primer dimers) by multiplying each data point of Figure 57 with the ratio of intended product to total product determined by melting peak integration. *See* Wittwer, page 93, line 23 through page 94, line12.

Lastly, at the bottom of page 3 of the instant Office Action, the PTO alleges that page 59, lines 1-15 of Wittwer teach the calculation of amplification efficiency "from the function determined in step e)." Applicants submit that because Wittwer requires amplification efficiency to be independent of the amount of nucleic acid, Wittwer cannot teach or suggest a method of determining amplification efficiency as a function of the amount of a target nucleic acid as recited in Claim 1. The section cited by the PTO teaches that within the log-linear portion of each curve, fluorescence can be determined by the formula: $F=AN(1+E)^n$, where F is the fluorescence signal, A is a fluorescence scaling factor, N is the starting copy number, E is the amplification efficiency, and n is the number of cycles. *See* Wittwer, page 59, lines 3-7. Wittwer further states that "A and E could first be determined for values of N that bracket the unknown. Then, the best value of N for the unknown could be determined." *See* Wittwer, page 59, lines 7-9. By calculating E for two N values that bracket the unknown, and using the value of E thus determined to calculate the unknown N, Wittwer requires that E *not* depend on N. In Wittwer, amplification efficiency must be constant, not a function of the amount of target nucleic acid.

In contrast, according to Applicant's invention, amplification efficiency *is dependent* on the amount of target nucleic acid and is, in fact, determined as a function of the amount of target nucleic acid. *See* Claim 1, step (e). By teaching that amplification efficiency is a constant and is *not dependent* on the amount of target nucleic acid, Wittwer in fact *teaches away* from Applicant's claims. Applicants remind the Examiner that teaching away is indicative of non-obviousness. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.* 220 USPQ 303 (Fed. Cir. 1983); MPEP § 2141.02; *In re Hedges* 228 USPQ 685 (Fed. Cir. 1986); MPEP § 2145.

Moreover, Claim 1 recites, *inter alia*, determining the cycle number at which a threshold is exceed for each dilution in a series to determine amplification efficiency. Wittwer does not teach or suggest determining the cycle number at which a threshold is exceeded for each dilution in a series. Nor does Wittwer even mention determining amplification efficiency in conjunction with the thresholds. Wittwer may mention the determination of a cycle number at which a particular signal threshold value is exceeded, however, Wittwer does not connect the determination of the cycle number to the determination of amplification efficiency as is done in Claim 1. Instead, Wittwer uses the

cycle number to *quantify* the amount of nucleic acid by threshold or endpoint interpolation. *See* Wittwer, Figure 22 and page 57, line 32 through page 58, line 16.

Brown does not remedy the many deficiencies of Wittwer. First, although Brown might teach preparation of a dilution of the target nucleic acid, Brown does not teach a method of determining amplification efficiency as a function of the amount of target nucleic acid. Nor does Brown teach a method of determining amplification efficiency in conjunction with fluorescence thresholds. In fact, Brown does not teach *any* method of determining amplification efficiency. Instead, Brown discloses methods "for determining the existence and/or initial concentration of a target nucleic acid in samples of about 1 microliter or less." *See* Brown, column 4, lines 64-67. In one embodiment, the method is carried out "by conducting replicate polymerase chain reactions on a set of terminally diluted or serially smaller samples and counting the number of positive polymerase chain reactions yielding specific product." *Id.* at column 5, lines 38-43. Thus, like Wittwer, Brown does not teach a method of determining amplification efficiency as a function of the amount of target nucleic acid, nor does it teach a method of determining amplification efficiency based on the determination of the cycle number at which a defined signal threshold value is exceeded. Applicants therefore submit that Wittwer and Brown, alone or in combination, do not teach each and every element of independent Claim 1.

Further, Applicants respectfully submit that the PTO has failed to provide any suggestion or motivation to combine Wittwer and Brown. The PTO asserts that "it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to substitute and combine the preparation of a dilution of the target nucleic acid . . . of Brown *et al.* in the method of sampling, amplifying and quantifying segment of nucleic acid of Wittwer *et al.*" Applicants respectfully remind the Examiner that in order to make out a *prima facie* case of obviousness there must be some suggestion or motivation, either in the references or in the knowledge available to one of ordinary skill in the art to modify the references or to combine the reference teachings and that there must be some reasonable expectation of success. The teaching or suggestion and the expectation of success must both be found in the prior art and not based on Applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ.2d 1438 (Fed. Cir. 1991). Applicants submit that, contrary to what the PTO asserts, the PTO has provided no motivation to combine Wittwer and Brown.

The PTO cites Brown, column 4, lines 23-26 as "strong motivation" to combine Wittwer and Brown. The cited lines state that there is a need for performing multiple different amplification and detection reactions in parallel on a single specimen and for economizing usage of reagents in the process. Applicants submit that the lines cited by the PTO is the motivation provided in Brown, based on the art discussed in its "Background of the Invention" section for its *own* invention. The motivation cited by the PTO is completely fulfilled by Brown itself. It is Brown, and not Wittwer that "perform[s] multiple different amplification and detection reactions in parallel on a single specimen." Applicants therefore submit that the reasons cited by the PTO would not motivate one of skill in the art to combine Brown with *any* other reference, much less Wittwer. Accordingly, Applicants submit that the Patent Office has failed to meet the burden of demonstrating motivation to combine the teachings of the references. Further, as discussed above, neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of Claim 1 or Claims 2-8 and 15 that depend therefrom.

1. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of Dependent Claims 2-8 and 15

Applicants respectfully submit that the references cited by the PTO are also not sufficient to establish a *prima facie* case of obviousness against dependent Claims 2-8 and 15 because neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of independent Claim 1 from which Claims 2-8 and 15 depend, nor do they teach several additional elements of Claims 2-8 and 15.

In addition to the elements of Claim 1, dependent Claims 2 and 3 recite that the function of Claim 1 that relates the copy number of the target nucleic acid to the cycle number where amplification exceeds a signal threshold is a non-linear, continuously differentiable function. Dependent Claims 4 and 5 recite that the amplification efficiency can be determined from the functions of Claims 2 and 3 as the negative local first derivative or the reciprocal negative local first derivative, respectively. Dependent Claims 6 and 15 recite that the function is determined with a polynomial fit, for example, of the 3rd, 4th, 5th, 6th or 7th degree.

Dependent Claims 7 and 8 recite a method for quantification of a target nucleic acid comprising, *inter alia*, determination of amplification efficiencies (as in Claim 1) of the target

nucleic acid and of a standard or reference nucleic acid; amplification and measurement of the amplification of the target nucleic acid and a standard or reference nucleic acid under similar conditions; and quantification of the target nucleic acid by correcting the measured amplification with the determined amplification efficiencies.

On page 3 of the instant Office Action, the PTO alleges that Wittwer teaches determining a non-linear continuously differentiable function of a logarithm of copy number and calculating the amplification efficiency from the function. The PTO cites Figure 22 and page 58, lines 1-4 and page 59, lines 1-15 as support for its allegations. While this issue is moot because the PTO has not made out a *prima facie* case of obviousness against independent Claim 1, Applicants submit that, contrary to what the PTO cites, Wittwer does not teach determining *any* function of a logarithm of copy number, much less a *non-linear continuously differentiable* function, or a method of calculating amplification efficiencies from such a function.

As discussed above, Figure 22 and the text on page 58, lines 1-4, do not teach or suggest a method for determining the efficiency of the amplification of a target nucleic acid. They teach endpoint *interpolation* to compare the fluorescence of the unknown with the standards at a given cycle number and threshold interpolation to determine the cycle number at which each curve exceeds a given fluorescence value. Furthermore, because Wittwer requires amplification efficiency to be independent of the amount of nucleic acid as discussed above, Wittwer teaches methods that use linear functions, not non-linear functions as recited by Claims 2 and 3. To illustrate, in Wittwer's formula $F=AN(1+E)^n$ cited by the PTO, the fluorescence signal, F, must depend *linearly* on the copy number, N, of the nucleic acid because Wittwer assumes that amplification efficiency, E, is constant. Wittwer's use of linear functions thus teaches away from Claims 2 and 3 that recite the use of non-linear functions.

Further, citing page 59, lines 1-15 of Wittwer, the PTO alleges that Wittwer teaches that the amplification efficiency is determined as the negative and reciprocal local first derivatives of continuously differentiable functions. *See* top of page 4 of the instant Office Action. Contrary to what the PTO states, Wittwer does not teach determining negative and reciprocal local first derivatives of continuously differentiable functions as recited in dependent Claims 3 and 4. In fact, page 59, lines 1-15 of Wittwer, or the entire publication,

does not even *mention* a negative or a reciprocal negative local first derivative or a continuously differentiable function.

Next, the PTO states that on page 59, lines 1-22, Wittwer teaches that the non-linear continuously differentiable function is determined with a polynomial fit of the third degree. Applicants would like to remind the PTO that a polynomial of the third degree is, for example, an equation which reads as follows: $F(x) = A_3x^3 + A_2x^2 + A_1x + A_o$, where A_3, A_2, A_1 and A_0 are coefficients of the various powers of the variable, x. Neither the lines of Wittwer cited by the PTO (page 59, lines 1-22), nor any other in the entire document refer to a function that is a polynomial of the third degree.

The PTO also alleges that Wittwer teaches a method for the absolute quantification of a target nucleic acid as recited by Claim 7. While Wittwer may teach *a* method for the absolute quantification of a target nucleic acid, it does not teach the method of Claim 7 which recites a method for absolute quantification of a target nucleic acid by determining the amplification efficiencies of target nucleic acid and an internal or external standard *as a function of the original amount of nucleic acid*, as is taught by independent Claim 1.

At the bottom of page 4 of the instant Office Action, the PTO alleges that Figure 59 of Wittwer teaches correction of copy number with amplification efficiencies. As discussed above, Figure 59 of Wittwer does not teach correction of copy number with amplification efficiencies. Instead, Figure 59 teaches a correcting abnormal or unexpected amplification by multiplication with a ratio of intended product to total product.

Brown does not remedy the deficiencies of Wittwer. Brown does not teach *any* method of determining amplification efficiency, let alone one where it is determined from a non-linear continuously differentiable function of a logarithm of copy number as the negative or the reciprocal negative local first derivative of the function. Also, Brown does not teach determining a non-linear continuously differentiable function with a polynomial fit of any degree. Further, Brown does not teach a method for absolute (or relative) quantification of a target nucleic acid by determining the amplification efficiencies of target nucleic acid and standard or reference nucleic acid as functions of the amount of nucleic acid.

Since neither Wittwer nor Brown, alone or in any combination, teaches or suggests a method of determining amplification efficiency of a target nucleic acid *as a function of the original amount of target nucleic acid* where, *inter alia,* a defined signal threshold is set and a

cycle number where amplification, as measured in real-time, exceeds the threshold is determined for each dilution in a series, the PTO's combination of references fails to teach or suggest each and every element of Claim 1. Claims 2-8 and 15 ultimately depend from Claim 1. The references are therefore not sufficient to establish a *prima facie* case of obviousness against Claims 1-8 and 15. Applicants respectfully request that the rejection of Claims 1-8 and 15 under 35 U.S.C. § 103(a) be withdrawn.

C. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of Claims 9-14 and 16-17

Applicants respectfully submit that the references cited by the PTO are not sufficient to establish a *prima facie* case of obviousness against Claims 9-14 and 16-17 because neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of Claims 9-14 and 16-17. As discussed in Section A above, in order to establish *prima facie* obviousness, the PTO must show that the prior art, either alone or in combination, teaches or suggests each and every limitation of the rejected claims. By failing to show such a teaching or suggestion in the art, the PTO has failed to establish *prima facie* obviousness against Claims 9-14 and 16-17.

Independent Claims 9 and 10 recite methods for quantification of a target nucleic acid relative to a reference nucleic acid standardized with a calibrator sample. The methods comprise the determining cycle numbers Cp at which signal thresholds are exceeded for dilutions of the target nucleic acid and the reference nucleic acid to determine continuously differentiable functions relating the Cp values to the logarithm of the amount of target or reference nucleic acid. The methods further comprise measuring the Cp values of the reference nucleic acid in a sample to be analysed and in a calibrator sample and using the continuously differentiable functions to calculate the ratio of target nucleic acid to reference nucleic acid.

As discussed in Section B above, Wittwer teaches away from methods using non-linear functions such as those recited in Claims 9 and 10. Wittwer instead requires that amplification efficiency be constant and that amplification depend linearly on the amount of nucleic acid. Furthermore, Wittwer teaches or suggests nothing about the use of a calibrator sample to correct the relative amount of a target nucleic acid to a reference nucleic acid. Applicants simply do not understand the PTO's reference to Wittwer's "inherent" teaching.

Applicants respectfully draw the PTO's attention to the fact that there is no mention of a reference/standard nucleic acid, a calibrator sample or a quotient of any kind anywhere in Wittwer.

Brown does not remedy the many deficiencies of Wittwer. As discussed in Section B above, Brown does not teach or suggest that amplification efficiency depends on the amount of a nucleic acid and does not teach or suggest the use of non-linear functions as recited in Claims 9 and 10. Furthermore, Applicants submit that Brown, like Wittwer, also does not teach or suggest the use of a calibrator sample to correct the relative amount of a target nucleic acid to a reference nucleic acid. Accordingly, Applicants submit that neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of independent Claims 9 and 10.

The PTO has further alleged that Wittwer and/or Brown teach some elements of dependent Claims 11-14 and 16-17. Applicants remind the PTO that this issue is moot because the PTO has not made out a *prima facie* case of obviousness against independent Claims 9 and 10. Nevertheless, Applicants submit that, contrary to what the PTO alleges, neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of dependent Claims 11-14 and 16-17.

Claims 11 and 16 further recite that the functions of step (e) are determined with a polynomial fit, for example, of the 3rd, 4th, 5th, 6th or 7th degree. As discussed in Section B, above, neither Wittwer nor Brown, alone, or in combination, teaches or suggests a function that is a polynomial of any degree.

Since neither Wittwer nor Brown, alone or in any combination, teaches or suggests, for instance, the use of non-linear functions or the use of a calibrator sample as recited in Claims 9 and 10, the PTO's combination of references fails to teach or suggest each and every element of independent Claims 9 and 10. Since Claims 11-14 and 16-17 ultimately depend from Claims 9 and/or 10, the combination of references is also not sufficient to establish a *prima facie* case of obviousness against Claims 11-14 and 16-17. Applicants respectfully request that the rejection of Claims 9-14 and 16-17 under 35 U.S.C. § 103(a) be withdrawn.

D. Claims 1-17 Satisfy 35 U.S.C. § 103

It is respectfully submitted for the reasons given above that the PTO has not established a *prima facie* case of obviousness against Claims 1-17. Applicants respectfully request that the PTO withdraw the rejection under 35 U.S.C. § 103 of Claims 1-17.

E. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of New Claims 18-35

Applicants respectfully submit that the references cited by the PTO are not sufficient to establish a *prima facie* case of obviousness against new Claims 18-37 because neither Wittwer nor Brown, alone or in combination, teaches or suggests each and every element of Claims 18-35.

Independent Claim 18 recites a method of determining amplification efficiency of a nucleic acid as a function of concentration comprising, *inter alia,* determining a cycle number for each different dilution of the nucleic acid where amplification exceeds a threshold. Claims 19-30 depend from Claim 18. As discussed in Section B above, because neither Wittwer nor Brown, alone or in any combination, teaches or suggests a method of determining amplification efficiency of a nucleic acid *as a function of the original amount of target nucleic acid* where, *inter alia,* a cycle number where amplification, as measured in real-time, exceeds a threshold is determined for each different dilution of nucleic acid, the PTO's combination of references fails to teach or suggest each and every element of Claim 18. Since Claims 19-30 ultimately depend from Claim 18, the references are therefore also not sufficient to establish a *prima facie* case of obviousness against Claims 19-30.

Independent Claim 31 recites a method for quantification of a target nucleic acid relative to a reference nucleic acid comprising, *inter alia*, generating a continuously differentiable target function, F_T, of target nucleic acid cycle number and a continuously differentiable reference function, F_R, of reference nucleic acid cycle number wherein, F_T maps the cycle numbers at which threshold amplification is exceeded for different dilutions of the target nucleic acid to the logarithm of concentration of the target nucleic acid and F_R maps the cycle numbers at which threshold amplification is exceeded for different dilutions of the reference nucleic acid to the logarithm of concentration of the reference nucleic acid. As discussed in Sections B and C above, because neither Wittwer nor Brown, alone, or in

combination, teaches or suggests, for example, a method for quantifying a target nucleic acid relative to a reference nucleic acid by generating continuously differentiable target and reference functions that map the cycle numbers at which threshold amplification is exceeded for different dilutions of the target and reference nucleic acids to the logarithm of concentration of the target and reference nucleic acids, respectively, the PTO's combination of references fails to teach or suggest each and every element of independent Claim 31. Since Claims 32-37 ultimately depend from Claim 31, the references are therefore also not sufficient to establish a *prima facie* case of obviousness against Claims 32-37.

In view of the foregoing, Applicants respectfully submit that new Claims 18-37 meet the requirements for patentability under 35 U.S.C. § 103.

CONCLUSION

Applicants respectfully contend that all grounds for rejection have been overcome and/or obviated by the amendments and remarks set forth herein, and that Claims 1-37 are in condition for allowance. Accordingly, the PTO is respectfully solicited to allow claims. If any issues remain in connection herewith, the PTO is invited to telephone the undersigned to discuss same.

No fees in addition to the extension fee are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 CFR § 1.17 and all required extension of time fees, or credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150.

Respectfully submitted,

Dated: December 4, 2002

Rahul Pathak (Reg. No. 42,983)

For Nikolaos C. George (Reg. No. 39,201)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosures

EXHIBIT A
MARKED UP VERSION OF AMENDED PARAGRAPHS

In paragraph 194 of U.S Publication No. 2002/0058262 A1, please replace original formula 6 with new formula 6 as follows:

$$\frac{\frac{N(T)_{0A}}{N(R)_{0A}}}{\frac{N(T)_{0K}}{N(R)_{0K}}} = \frac{\dfrac{[N(R)_{nA}]\underline{N(T)_{nA}} * \prod_{1-n} E_A(R_X)}{[N(T)_{nA}]\underline{N(R)_{nA}} * \prod_{1-n} E_A(T_X)}}{\dfrac{[N(R)_{nK}]\underline{N(T)_{nK}} * \prod_{1-n} E_K(R_X)}{[N(T)_{nK}]\underline{N(R)_{nK}} * \prod_{1-n} E_K(T_X)}}$$

EXHIBIT B

MARKED-UP VERSION OF AMENDED CLAIMS

1. (Twice Amended) A method for determining the efficiency of an amplification of a target nucleic acid comprising the steps of:

a) preparing a dilution series of the target nucleic acid [is prepared];

b) amplifying the target nucleic acid [is amplified] under defined reaction conditions and measuring the amplification [is measured] in real-time;

c) setting a defined signal threshold value [is set];

d) determining, for each dilution, the cycle number [is determined] at which the signal threshold value is exceeded; and

e) determining the amplification efficiency [is determined] as a function of the original amount of target nucleic acid.

2. (Twice Amended) The method of claim 1, further comprising [wherein the efficiency of an amplification is determined by:

a) preparing a dilution series of the target nucleic acid;

b) amplifying the target nucleic acid under defined reaction and the amplification of the nucleic acid being measured in real-time;

c) setting a defined threshold value;

d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

e)] determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and

[f)] calculating the amplification efficiency [E from the function determined in step e)] from said non-linear continuously differentiable function.

3. (Twice Amended) The method of claim 1, further comprising [wherein the efficiency of an amplification is determined by:

a) preparing a dilution series of the target nucleic acid;

b) amplifying the target nucleic acid under defined reaction, the amplification of the nucleic acid being measured in real-time;

c) setting a defined threshold value;

d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

e)] determining a non-linear continuously differentiable function of the cycle number determined in step d) as a function of the logarithm of the copy number of target nucleic acid used [in each case] for the amplification; and

[f)] calculating the amplification efficiency [E from the function determined in step e)] from said non-linear continuously differentiable function.

7. (Amended) A method for [the] absolute quantification of a target nucleic acid in a sample comprising the steps of:

a) [determination of] determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions as claimed in claim 1;

b) [amplification of] amplifying the target nucleic acid contained in the sample and of the internal or external standard under the same defined reaction conditions;

c) [measurement of] measuring the amplification of the target nucleic acid and standard in real time; and

d) [calculation of] calculating the original copy number in the sample by [correction of] correcting the copy number derived from step c) with [the aid of] the amplification efficiencies determined in step a).

8. (Twice Amended) A method for quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:

a) [determination of] determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions as claimed in claim 1;

b) [amplification of] amplifying the target nucleic acid contained in the sample as well as of the reference nucleic acid contained in the sample under the same defined amplification conditions;

c) [measurement of] measuring the amplification of the target nucleic acid and of the reference nucleic acid in real time; and

d) [calculation of] calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by [correction of] correcting the ratio derived from step c) with [the aid of] the amplification efficiencies determined in step a).

9. (Twice Amended) A method for [relative] quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) [preparation of] preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) [amplification of] amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids [acid being measured] in real-time;

c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;

d) determining the cycle [number] numbers Cp [to] at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;

e) determining a continuously differentiable function of the Cp values determined in step d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the [determined] Cp values determined in step d) as a function of the logarithm of the amounts used of reference nucleic acid;

f) [determination of] determining the Cp values of the target nucleic acid and reference nucleic acid in [the] a sample to be analysed as well as in a calibrator sample;

g) [assignment of] assigning the Cp values measured in step f) to [a] particular [function] values of the functions determined in step e);

h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and

i) [determination of] determining the ratio of the two quotients from step h) as a measure [for] of the original amount of target nucleic acid contained in the sample to be analysed.

10. (Twice Amended) A method for [relative] quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) [preparation of] preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) [amplification of] amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids [acid being measured] in real-time;

c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;

d) determining the cycle [number] numbers Cp at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;

e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the Cp values determined in step d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the [determined] Cp values determined in step d);

f) determining the Cp values of the target nucleic acid and reference nucleic acid in [the] a sample to be analysed as well as in a calibrator sample;

g) [assignment of] assigning the Cp values measured in step f) to particular [function] values of the functions determined in step e);

h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and

i) [determination of] determining the ratio of the two quotients from step h) as a measure [for] of the original amount of target nucleic acid contained in the sample to be analysed.

12. (Twice Amended) The method of claim 10, wherein the amplified nucleic acids are detected with at least one [fluorescent-labelled] fluorescently-labeled hybridization probe.

13. (Twice Amended) The method of claim 12, wherein the amplified nucleic acids are detected with [of] FRET hybridization probes, molecular beacons, or [TaqMan®] TAQMAN® probes.

17. (Amended) The method of claim 14, wherein said DNA-binding dye is [Sybr®] SYBR® Green I.